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(54) Method of diagnosis and treatment of leprosy utilizing lymphokines.

(57) The implication of lymphokines in the body's defense mechanism against leprosy makes them suitable pharmaceutical agents to combat this disease. Either IL-2 or -interferon, separately or together may be administered as a therapeutic agent to an indivdiual suffering from leprosy. When used as anti-leprosy agents the lymphokines may be administered topically at sites of injection or may be administered systemically.

It has been found that peripheral blood mononuclear cells from 10 out of 10 lepromatous (LL) patients displayed no release of γ-IFN upon stimulation by the specific antigen. In contrast, 6 out of 6 tuberculoid (TT) patients, and borderline tuberculoid (BT) had strong γ-IFN responses. The low responsiveness to antigen of some of the borderline lepromatous cases (BL) case suggests that this assay can be helpful in evaluating the prognosis of the disease, suggesting which patients display suppression of cell-mediated responses and would tend to follow the lepromatous course.

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This invention concerns a more meaningful functional assay for the diagnosis and improvement of the immunological status of patients with leprosy with the lymphokines gamma interferon and interleukin II.

Field of Invention

Leprosy is a chronic disease caused by Mycobacteria leprae. In some subjects, the disease is disseminated (lepromatous form). In these, widespread lesions present lose infiltrates and the organisms are found in large numbers in skin macrophages. Such individuals have a predominant role in transmitting the disease. The rapid progression of this type of lesion and their heavy bacillary load has been ascribed to deficient T-cell response to the specific antigen, M. leprae, but not to other antigens (Turk, J.L. and Bryuson, A.D.M. Adv. Immunol. 13: 209-266 (1971); Myrvang, B., Godal, T., Ridley, D.S., Froland, S.S., and Song, Y.K. Clin. Exp. Immun. 14: 541-553 (1973)). In contrast, subjects with the less severe form of the disease (tuberculoid) develop strong cell-mediated immunity that results in effective killing of bacteria inside macrophages. T-cells from these patients respond strongly to M. leprae antigen.

Studies of the lesions of leprosy subjects has revealed that in the lepromatous cases of leprosy the cellular infiltrates are devoid of "helper" T-cells, as identified by OKT4/Leu 3a monoclonal antibodies. This is in contrast to tuberculoid patients where a predominance of "helper" T-cells is found (Van Voorhis, W.C., Kaplan G., Sarno, E.N., Horwitz, M.A., Steinman, R.M., Lewis, W.R., Nogueira, N., Hair, L.S., Gattass, C.R., Arrick, B.A. and Cohn, Z.A. New England J. Medicine 307:1593-1597 (1982).

In the process of macrophage activation, helper-type T-cells generate factors ("lymphokines") which lead to functional changes in macrophage function leading to a microbicidal state (Nogueira, N., Gordon, S. and Cohn Z. J. Exp. Med. 146:172 1977; Nogueira, N. and Cohn, Z.A. J. Exp. Med. 148: 288 1978; Nogueira, N., Chaplan, S. Reesink, M., Tydings J. and Cohn, Z.A. J. Immunol. 128: 2142 1982). Among other soluble factors, γ-interferon seems to be a large component of such "lymphokine" preparations (Waksman, B.H. Biology of the Lymphokines S. Cohen, E. Pck and J.J. Oppenheim, editors Academic Press, New York 585-616 1979). γ-interferon has been shown in a tumor cell mode to copurify with a "macrophage activating factor" (Pace, J.L., Russel, S.W., Schneider, R.D., Altman, A. and Katz, D.H. P.N.A.S. 3782-3786 1983; Wisseman, C.L. Jr. and Waddell, A. J. Exp. Med. 1780-1993 1983).

Recent studies have shown that T-cells that have been "primed" with antigen can be induced to continuous proliferation in vitro by exposure to medium containing IL-2 (Gillis, S. and Smith K.A. Nature 268, 154-156 1977; Alvarez, J.M., Londazuri Mo de, Bonnard, G.D. and Hekerman, R.B. J. Immunol. 121: 1270-1275 1978; Kurnik, J.T., Hayward, A.R. and Altevogt, P. J. Immunol. 126: 1307-1311 1981). Patients with lepromatous leprosy have been shown to have their cell-proliferation capability restored in response to the specific antigen M. leprae, when cultured in IL-2 rich media (Haregeworn, A., T. Godal, Mustata, A.S., Belehu, A., Yemanebernan, T. Nature 303: 342 1983). This suggests that the defect in lepromatous leprosy patients is due to deficiency in release of IL-2 or other related mediators rather than a lack of responsive T-cells.

Accordingly, the role of γ-interferon and interleukin II in the progress of leprosy has been studied. Specifically, peripheral blood mononuclear cells from patients with both lepromatous and benign tuberculoid leprosy were examined, in the presence of M. leprae antigen and mitrogens such as Conconavalin A, in terms of capacity for γ-interferon release. The effect of IL-2 on release of γ-interferon was also examined.

DETAILS

Patients with leprosy were examined for the capacity of their peripheral blood mononuclear cells to respond to M. leprae antigen and to mitogens, such as concanavalin A in terms of γ-interferon and IL-2 release. γ-interferon was measured by anti-viral activity in human foreskin cells infected with encephalo-myocarditis (EMC) virus; γ-interferon was identified by the use of a γ-specific rabbit antibody which specifically neutralized γ-interferon activity.

Table I summarizes these results:

Table I

-IFN Release by RBMC of Leprosy Patients

Patients	Treatment	Clinical Diagnosis	Hist. Diagnosis	-IFN (U/ml) M. Leprae	-IFN (U/ml) Con A
SRG	None	L	LL	0	0
EAS	None	L	LL	0	0
AA	1 yr.	L	LL	0	0
RM	3 yr.	L	LL	16	32
FEG	1 yr.	L	LL	8	8
CL	2 yr.	L	LL	0	16
APA	None	L	LL	0	0
AL	1 yr.	L	LL	0	0
CBS	None	L	BL	0	0
SS		T	BB	64	16
ML		T	BT	32	64
RCMP		B	BT	128	512
RS		T	BT	128	128
MV		T	BT	256	ND
BEBS		T	TT	256	256

The ability of lepromatous patient's mononuclear cells to release γ-IFN in response to the specific antigen in the presence and absence of a purified preparation of human IL-2 was tested. Table II summarizes these results. IL-2 indeed restored the response of these cells to the specific antigen in terms of release of γ-IFN.

Table II

Restoration of γ-IFN release responsiveness by IL-2.

Patients	Treatment	Clinical Diagnosis	Histopatological Diagnosis	-INF -IL-2	+IL-2 1U/ml	+IL-2 5U/ml
FEG	1 yr.	L	LL	8	128(16)	ND
RM	3 yr.	L	LL	16	128(8)	ND
CL	2 yr.	L	LL	16	128(32)	512(32)
AL	1 yr.	L	LL	0	0(0)	16(8)
EG	1 yr.	L	LL	0	32(0)	64(32)

( ) IL-2 + cells no Ag

The suggestion that the lepromatous lesions contain such heavy bacillary load due to deficiency of either or both of these mediators leads to the attempt to try either the natural or cloned mediators in patients with the severe form of the disease, first by local inoculation in isolated lesions and later by the systemic route.

What is Claimed:

1. γ-IFN as an anti-leprosy agent.

2. IL-2 as an anti-leprosy agent.

3. Method of treating leprosy in an individual comprising administering to said individual an anti-leprosy effective amount of γ-IFN and IL-2, separately or together.

4. Method of Claim 3 wherein said γ-IFN and IL-2 are administered systemically.

5. Method of Claim 3 wherein said γ-IFN and IL-2 are administered topically.

6. Diagnostic test for leprosy in an individual comprising:
   a) contacting peripheral blood mononuclear cells from the individual with an antigen or mitogen; and
   b) measuring γ-IFN released from said cells.

7. Test of Claim 6 wherein said antigen is M. leprae.

8. Test of Claim 6 wherein said mitogen is Conconavalin A.